EXHIBIT A41

POTENTIAL DELIVERY OF CONTRACEPTIVE AGENTS TO THE FEMALE REPRODUCTIVE TRACT

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INTRODUCTION

Vaginal contraceptives have played a positive role in fertility planning for years. Prior to the introduction of intrauterine devices (IUDs) and oral contraceptives (OCs), jellies, creams, and suppositories were used, sometimes in conjunction with the diaphragm or condom, but usually they served as the sole barrier.[1] The higher failure rates of the vaginal contraceptives in relation to other contraceptive devices (IUDs and OCs) were believed to be due primarily to the inconsistent use of the method rather than to failure of the method during use.

Disadvantages cited with IUDs include: (a) increased menstrual bleeding and spotting; (b) expulsion soon after insertion; (c) unwanted pregnancies that are more likely to be ectopic; and (d) increased frequency of pelvic infection.[2] Problems noted with OCs have included: (a) the requirement for daily administration; (b) the subsequent daily variation in blood steroid concentrations; and (c) association with circulatory system diseases.[3]

OCs possess two distinct advantages over other fertility control methods. They are the most effective method of preventing pregnancy currently available, and they facilitate more satisfactory sexual relations.[3] However, vaginal contraceptives have been of continuing interest because of some of the disadvantages associated with OCs and IUDs and because recent clinical data have shown that the newer preparations (e.g., foams in aerosol and pressurized containers) can provide substantial protection if used correctly and regularly.[1]

D. H. Lewis (ed.), *Controlled Release of Pesticides and Pharmaceuticals*

Increased effort is being placed on improving the present status of vaginal antifertility agents with regard to their contraceptive efficacy, mode of administration, and general aesthetic acceptability. An approach which we believe has some promise in this regard is a self-administered microcapsule drug-delivery system for the female reproductive tract.

The primary factors affecting the rate of sperm transport through the reproductive tract include (a) muscular activity, (b) composition and physical characteristics of the fluids, (c) female orgasm, and (d) directional swimming activity of the spermatozoa.[4] The last factor may be of importance in assisting gamete movement past the cervical canal and in fertilization of the egg. However, passive sperm transport is indicated by (a) the rapid transport of sperm to upper segments of the reproductive tract in various animals, including humans, (b) by the passage of seminal plasma constituents, dead sperm, and inert material following vaginal insertion in animals, and (c) the inability of sperm to cover that distance in so short a time period, if left to their normal swimming progression rates.[5]

The concept of a particulate drug-delivery system is further supported by studies in humans, which demonstrate the movement of inert particles through the reproductive tract. Following placement in either the vagina, cervix, or uterus, particles such as carmine or carbon black have been observed to migrate into the fallopian tubes or peritoneal cavity.[6,9]

The advantages of a microcapsular dosage form as a drug-delivery system might include the following: (1) flexibility in controlling the rate of drug delivery from the microcapsules by alteration of microcapsule size, membrane polymer, and wall thickness; (2) the potential for encapsulating antifertility drugs or therapeutic agents separately, but administering them simultaneously, and (3) the possibility of modifying the membrane surface properties or microcapsule size to permit pretargeting of the microcapsules to specific areas of the reproductive tract.

METHODS AND MATERIALS

To determine the feasibility of using a microcapsule drug-delivery system for the female reproductive tract, studies were undertaken to determine the fate of microcapsules following insertion in the vagina of stumptail monkeys (21 experiments) or baboons (10 experiments) at different phases of the menstrual cycle. To follow migration of the dosage form in the primate reproductive tract, tracer microcapsules (containing either soluble ^{125}I-human serum albumin or ^{85}Sr-micropheres) were monitored using a well-collimated detector system (Figures 1, 2, and 3). The collimator

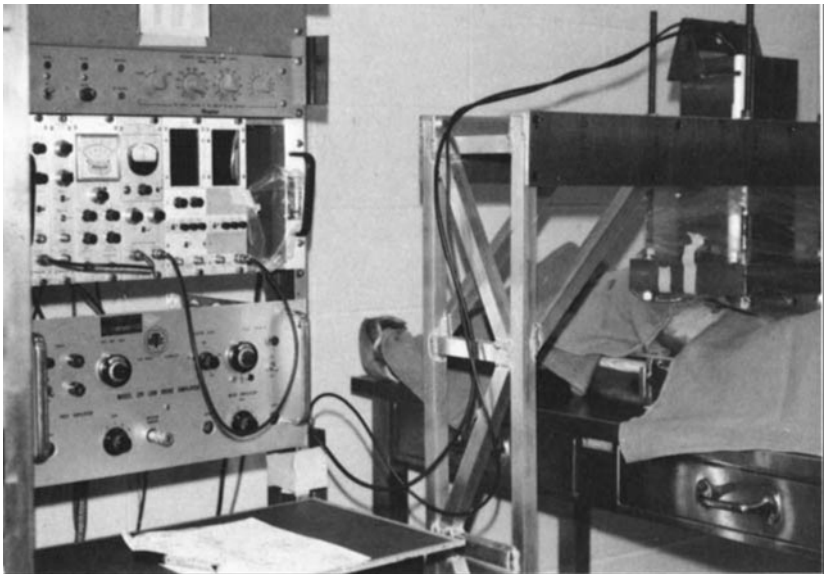

Figure 1. Physical set-up for monitoring distribution of microcapsules.

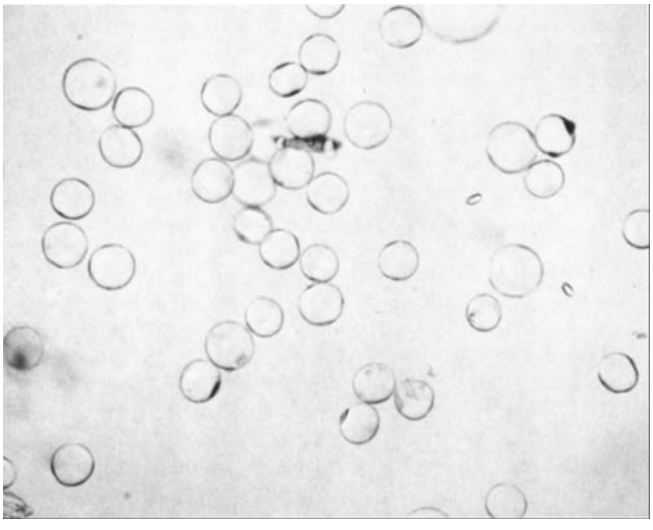

Figure 2. Tracer microcapsules containing ^{125}I-human albumin. Capsules are 50±10 µm diameter. Magnification = 81X.

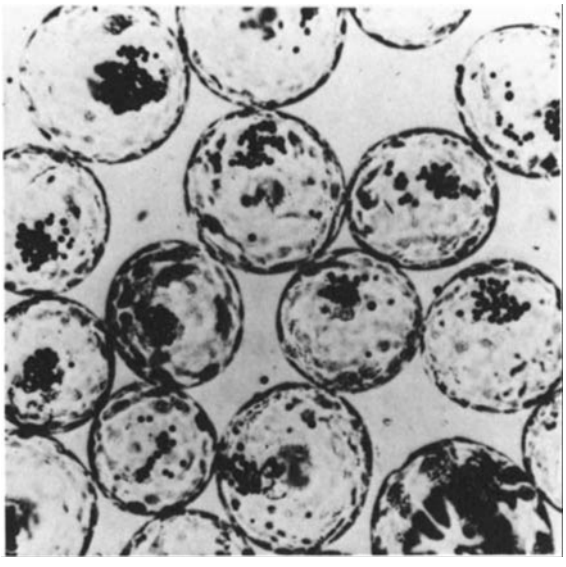

Figure 3. Tracer microcapsules containing ^{85}Sr-microspheres (15±5 μm). Magnification = 51X.

consisted of a 1/2-inch diameter aperture through five inches of lead. A 2-inch NaI crystal and photomultiplier tube were connected to a Hamner detection system. Discrimination and window settings were selected to optimize counting efficiency and minimize background. The collimator detected a 1-1/2-inch diameter circle at the plane of the primate's reproductive tract.

Animals were sedated with Sernylan® (phencyclidine hydrochloride, Bioceutic Laboratories, Inc., St. Joseph, MO), and the location of the reproductive organs (i.e., vagina, cervix, and uterus) was determined by rectal palpation. The animal's abdomen was then demarcated into grids (grids consisted of five 1/2-inch diameter circles) centered over the vagina, cervix, or uterus. Approximately 1 gram of tracer microcapsules containing 2-3 μCi of radioactivity was then inserted in the vaginal canal so that peak counts were obtained in the center circle of the vaginal grid. Even with this amount of radioactivity, only 0.2-0.4 percent of the counts could be detected following insertion at zero-time. This low percentage of detection was due to the high degree of collimation necessary to differentiate the reproductive-tract segments. All grids were then counted at zero-time and at predetermined times thereafter. Total counting time for each grid was five minutes. The tracer microcapsules were placed in the primate's vagina by either placing the tracer microcapsules in a frozen or unfrozen plug (approximately

one inch). The plug (capsules were packed in hollow glass tubing (1/4-inch O.D. x 7 inches)) was delivered by a solid-glass plunger.

A secondary study involved extirpating the reproductive tract in a single baboon to obtain a more accurate assessment of tracer microcapsule distribution following insertion in the vagina.

A mixture (0.3 g each) of two tracer capsule types (^{125}I and ^{85}Sr) was used to determine if a particular capsule diameter would migrate preferentially. The ^{125}I capsules were 20-37 μm in diameter and the ^{85}Sr capsules were 297-420 μm in diameter. An unfrozen plug (containing both capsule types homogeneously dispersed) was placed in the baboon's vagina near the external os of the cervix. The animal was in the early pre-ovulatory phase of its menstural cycle (12 days postmenses) and was kept sedated in a horizontal plane during the course of the experiment.

The animal was sacrificed at the end of six hours, and the reproductive tract was removed in toto. Each area of the reproductive tract (i.e., vagina, cervix, uterus, and fallopian tubes) was sectioned transversely into approximately 1/4-inch serial strips and counted simultaneously on a dual-channel counter for ^{125}I and ^{85}Sr by standard gamma counting techniques.

RESULTS

Stumptail Monkey Studies

The results obtained in stumptail monkeys are presented in Figures 4 and 5. Figure 4 illustrates studies with pre-ovulatory phase animals (Days 1-9, with the initiation of menses constituting the first day), while Figure 5 summarizes the results obtained from animals during the mid-cycle phase.

Capsule retention in the vaginal grid area of pre-ovulatory animals (Figure 4) averaged approximately 70 percent for capsule diameters larger than 200 μm in five experiments. However, for capsules between 63-200 μm in diameter, an average retention of 25 percent was obtained in the vaginal grid area in six experiments.

Transcervical migration from the vagina to the uterus (24 hours post-insertion) was observed to some degree in six out of eleven animals. In these studies it appeared that capsule diameters less than 300 μm in diameter showed preferential migration. However, one animal out of three at the largest capsule diameter did show migration of greater than three percent of the inserted microcapsules.

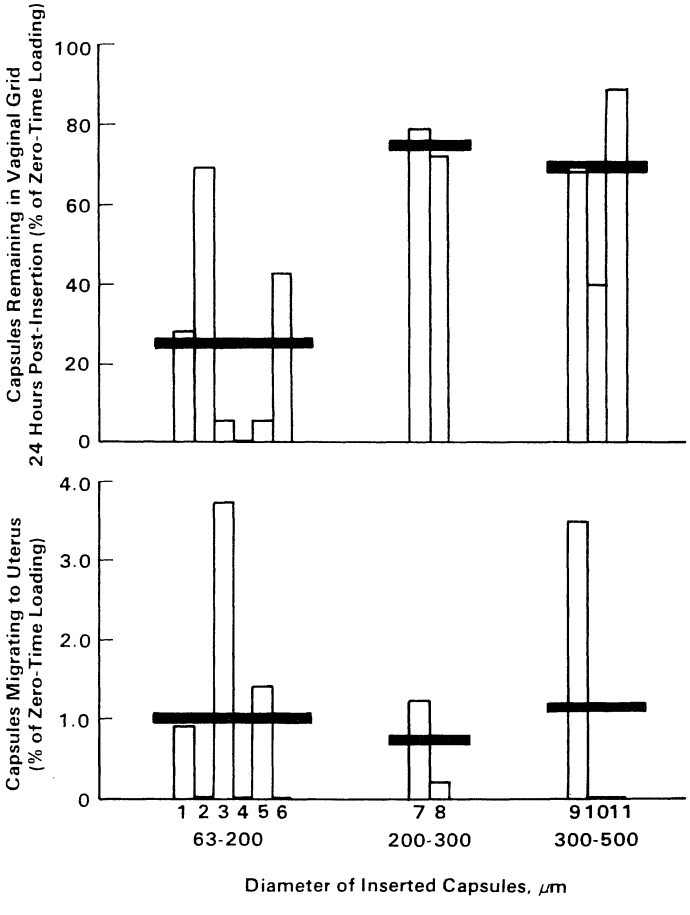

Figure 4. Distribution of microcapsules in stumptail monkeys (preovulatory phase).

In the studies with mid-cycle animals (Figure 5), both the percentage of capsules remaining in the vaginal grid (24 hours post-insertion) and the amount migrating into the uterus decreased with increasing capsule diameter.

In the limited number of studies conducted, the average percent of capsules remaining in the vaginal grid after 24 hours was much lower for the mid-cycle studies (22.1 percent) than for the pre-ovulatory studies (44.7 percent). The difference between the two menstrual phases was also reflected in a similar difference in transcervical migration. In the pre-ovulatory phase studies, the

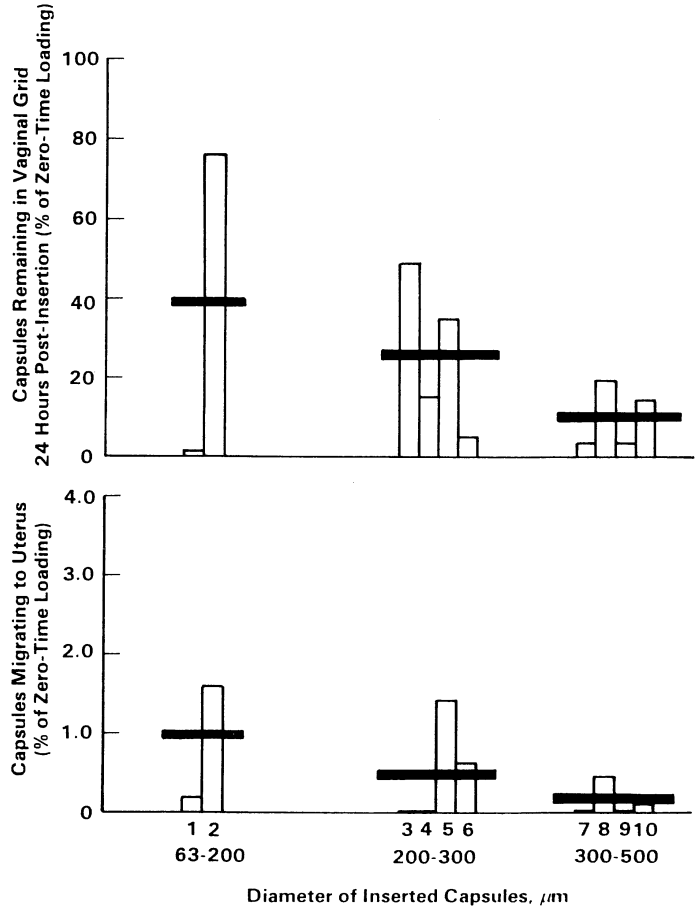

Figure 5. Distribution of microcapsules in stemptail monkeys (mid-cycle phase).

overall transcervical migration percentage was 0.98; for the mid-cycle phase studies, 0.42 percent was observed in the uterus after 24 hours.

Baboon Studies

Microcapsule distribution in the baboon reproductive tract is presented in Table I. In these studies, the percentage of capsules detected in the vaginal grid after 24 hours was much lower

TABLE I. SUMMARY OF TRANSCERVICAL MIGRATION STUDIES IN BABOONS

Capsule Diam., μm	Menstrual Phase	Percent Remaining in Vaginal Grid Area 24 Hours Post Insertion	Percent Transcervical Migration 24 Hours Post Insertion
63-105[a]	Early Follicular	1.5	0.0
63-105[a]	Early Follicular	0.0	0.0
63-105[a]	Early Follicular	0.0	0.0
63-105[b]	Midfollicular	3.8	0.0
105-210[b]	Preovulatory by 1-2 Days	4.2	0.0
105-210[b]	Preovulatory by 1-2 Days	3.1	0.0
105-210[b]	Preovulatory by 1-2 Days	2.9	0.57
105-210[b]	Early Luteal	30.8	0.75
105-210[b]	Early Luteal	4.8	2.42
297-420[b]	Preovulatory by 304 Days	6.0	0.0

[a] Baboons kept anesthetized for only first hour.

[b] Baboons kept anesthetized for first six hours.

than in comparable stumptail monkey experiments. However, when the animals were kept anesthetized and in a horizontal plane for six hours, the percentage remaining in the vaginal grid generally increased.

Only three out of ten animals exhibited any transcervical migration, with two of the three migration occurrences during the early luteal phase of the menstrual cycle.

The single experiment in which the baboon was sacrificed six hours after insertion of two different tracer microcapsules is summarized in Table II. These results indicate that (a) there is essentially no difference in transcervical migration between the two sizes, (b) migration occurs relatively rapidly (within six hours) into the cervix, uterus, and fallopian tubes, (c) almost 60 percent

TABLE II. PLACEMENT OF TWO DIFFERENT MICROCAPSULE SIZES IN A SINGLE BABOON

	Net CPM	
	^{125}I	^{85}Sr
Vagina	3,235,161	3,472,193
Cervix	22,648	27,078
Uterus	3,460	3,411
Fallopian Tubes	1,713	1,796

Total ^{125}I Net CPM Placed in Vagina = 5,929,837
Total ^{85}Sr Net CPM Placed in Vagina = 5,793,150

	Percentage of Migration After 6 Hours	
	^{125}I	^{85}Sr
Cervix	0.38	0.47
Uterus	0.05	0.06
Fallopian Tubes	0.03	0.03
Total	0.46	0.56

of the microcapsules were still present in the vagina at sacrifice. Capsules not accounted for in Table II may have been lost in the exudate observed around the vaginal exterior during removal of the reproductive tract.

This experiment also points out a problem with the use of the highly collimated, external-detector system used in the studies described above. When the baboon was sacrificed six hours after insertion of the capsules, 0.4 percent of the inserted capsules were detected in the extirpated cervix. However, external measurements taken six hours post-insertion but prior to sacrifice detected no capsules in the cervical grid. This observation demonstrates the generally low sensitivity of the external counting system, which was employed to gain adequate resolution between the three reproductive segments monitored.

DISCUSSION

These preliminary results in the primate confirm that microcapsules placed in the vagina do migrate across the cervix. Although the percentage of vaginal retention and transcervical migration was low and variable in these studies, the data suggest the possibility of developing a controlled-release contraceptive system for the female reproductive tract. Such a system could be based upon microcapsules for delivery of fertility-control or therapeutic

agents, especially if the required effective local dose is low (e.g., micrograms per day delivered locally to the uterus or cervix).

It is clear that contraceptive or therapeutic systems based upon the transcervical migration of drug-containing particles will require the controlled manipulation of several complex processes. The primary factors controlling the local rate of drug delivery at each reproductive site (i.e., vagina, cervix, uterus, or fallopian tubes) can be related as shown in equation (1).

$$LDR = M \times F \times R \quad (1)$$

where LDR is the desired local delivery rate (μg drug/day), M is the mass of drug-containing particles initially administered (g), F is the fraction of the administered capsules that migrate to the selected site, and R is the actual *in vivo* release rate of drug from the carrier particles (μg drug/g capsules/day).

Each of the independent factors (M, F, and R) is subject to unique sets of constraints and relationships which affect the actual local delivery rate. For example, M will probably be subject to an upper limit, based on constraints imposed by the vaginal vault and hygienic factors such as minimal leakage following insertion. In the studies described above, M was approximately one gram of wet or frozen capsules/treatment.

The fraction, F, of particles migrating to the target organ, which was studied in this program, appears to be controlled by many factors. These probably include the phase of the menstrual cycle in which the particles are administered, the fluid properties of the reproductive-tract contents, and the physical characteristics of the particles. It seems possible, also, that F could depend on the amount of carrier particles administered, M, and the method used for particle insertion.

The *in vivo* release rate of the contraceptive agent, R, is dependent upon the physical properties of the particles, including diameter, wall thickness, concentration of drug in the particles, solubility of the drug in the wall polymer, and the capsule interior fluid. Transcervical migration contraception efficacy is further complicated by the contribution of secondary drug release by particles that do not migrate to the target organ. Thus, determination of the therapeutic dosage must include the contribution of carrier particles at all sites of the reproductive tract.

Many aspects of a particulate delivery system still need to be addressed, including (1) determination of the final destiny of the carrier particles following insertion, (2) the local concentration of drug required at each site for effectiveness, (2) the effect

of menstruation, and (4) the duration of contraceptive effectiveness (e.g., Is a single administration adequate for only a few days surrounding ovulation or for a full menstrual cycle, or could it suffice as a post-coital contraceptive?). Nonetheless, in spite of the obvious complexity associated with the proposed microcapsule drug-delivery system, we believe that development of the proposed drug-delivery system could lead to a convenient, safe, and reliable contraceptive system.

ACKNOWLEDGEMENT

The investigations described in this report were supported by Contract No. N01-HD-1-2230 from the National Institute of Child Health and Human Development.

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